the ar/R filter. Based on transport analyses and molecular dynamics simulation, a model is proposed through which the alanine substitution results in both the selectivity for critical metalloid nutrients such as boric acid while simultaneously restricting water flow through the ar/R selectivity filter. A mechanism involving two different rotameric states of the conserved arginine residue in this selectivity region is proposed to be responsible for the water-tight character of the pore. (Supported in part by NSF grant 1121465).

2206-Pos Board B343

Mechanism of Proton Transport of the M2 Proton Channel Studied by Constant pH Molecular Dynamics

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The M2 protein of the influenza virus is a proton-selective homotetrameric channel. During virus entry, M2 is activated by the low pH of the endosome and transports protons into the virion, initiating viral uncoating. A histidine tetrad in the pore of the M2 transmembrane domain is responsible for pH activation and proton selectivity. A number of different mechanisms have been proposed for proton transport of M2 based on experimental and computational studies. To test these hypotheses, we applied the explicit-solvent continuous constant pH molecular dynamics method to study the pH-dependent conformational dynamics of M2 in explicit membrane. The calculated pKa values of the histidine tetrad are comparable to experimental values. The C-terminal openning of M2 became wider when pH was lowered. This work provides novel insight into the coupled protonation and conformational dynamics of the M2 proton channel.

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Proton Permeation in Ci-Hv1 Voltage-Gated Proton Channels occurs through a Proton Wire Involving Residues D160 and D222 and It is Modulated by N264

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Hv1 channels are integral membrane proteins with the capacity to selectively permeate protons in a voltage and pH-dependent manner. As Hv1 lacks a pore domain, permeation must occur through the voltage-sensing domain. Previous reports propose a permeation pathway consisting in a stable water wire which allows proton to permeate by means of a Grotthuss mechanism. Our molecular dynamics simulations do not support the formation of such stable water wire since it shows a dry zone around residue N264 in the wild type and in N264 mutants. Mutations of residues D222 and N264 affects single channel conductance (determined by non-stationary noise analysis) and selectivity, suggesting that both residues are involved in the permeation pathway. Quantum dynamics simulations performed in our model of the open Ci-Hv1 wt and in silico mutants suggest that permeation occur through a proton wire involving residues D160 and D222, a process modulated by N264.
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Free Energy Simulations of Ion Translocation through Voltage-Gated Proton Channel Hv1

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The human voltage-gated proton channel (hHV1) is a transmembrane protein that is responsible for the selective permeation of protons across cell membranes in nasal mucosa, sperm, and white blood cells. hHV1 is a four-helix bundle (S1-S4) with anionic Asp112 on S1 forming a salt bridge with cationic Arg residues on helix S4 in the narrow region of the pore [Kulleperuma et al., J. Gen. Physiol. 141, 445-465 (2013)]. Mutation of Asp112 to Val abrogates channel properties. Unexpectedly, replacing Asp112 by a smaller neutral residue such as Ser turns HV1 into an anion selective channel that conducts Cl-, as does the double mutant D112V-V116S [Musset et al., Nature 480, 273-277 (2012); Morgan et al., J. Gen. Physiol. 142, 625-640 (2013)]. Although HV1 and its mutants exhibit drastic differences in ion permeation, the molecular basis of proton selectivity in WT and anion selectivity in mutants remains unexplained.
As the first step towards elucidating the charge selectivity of HV1, we perform molecular dynamics simulations with umbrella sampling to compute the free energy profile for the translocation of Na^+ and Cl- ions through the pore of a homology model of HV1 and its mutants. The calculations are repeated in conformational states of the channel differing in the extent of hydration of the pore and in the relative arrangements of pore residues. Results show how ion solvation and electrostatic interactions with charged side chains in the pore lumen modulate the energetics of ion permeation in HV1 and suggest a structural basis for charge selectivity.

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Investigating the Potentiation Effect of 2-APB on CRAC Channels

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2-aminoethyldiphenyl borate (2-APB) elicits both potentiation and inhibition effect on Ca^{2+} influx via CRAC channels. In this study we focused on understanding the underlying mechanism of its potentiation effect. We identified one key residue which are just located in the pore region, plays a vital role in the potentiating effect caused by 2-APB. Mutation of this residue with small side chain such as C, A, G, completely eliminate the potentiating effect, while mutation with large side chain such as M and I could generate 2-APB induced potentiation current as WT. Our results imply that the potentiation effect caused by 2-APB might has a close relationship with the change in the pore diameter.

2210-Pos Board B347

Slo2.x Potassium Channels are Involved in the Regulation of Heart Mitochondrial Function

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Mitochondrial potassium channels (MKCs) are believed to be important in stress response in the heart. Volatile anesthetic preconditioning (APC) is a method of protecting the heart from ischemia-reperfusion injury which elicits evolutionarily-conserved protective signaling pathways that converge at the mitochondrial level. Work in *C. elegans* has focused attention on the *Slo2* gene product as a transducer of APC effects on hypoxic survival and recent data from our lab demonstrate that this protective role is conserved in mammals. *Slo2* in mammals has diverged into two paralogs, *Slo2.1* (KCNT2; Slick) and *Slo2.2* (KCNT1; Slack). These genes code for Na^+-activated K^+ channels and are highly expressed in brain, but their function in cardiomyocytes and/or mitochondria is unknown. Examination of these channels has been limited to pharmacologic profiling which is hampered by overlapping sensitivities and off-target effects of small molecules. Herein we employed novel genetic deletions of Slo2.1 and Slo2.2 double knockout, Slo2.x dKO, in mice to confirm the role of these potassium channels in APC and identify their role in endogenous cardiac mitochondrial function. Preliminary data obtained using Slo2.x dKO reveal novel metabolic and morphologic phenotypes, indicating a functional relationship between mitochondrial potassium channels and regulation of mitochondrial oxidative phosphorylation. These data demonstrate a role of the Slo2.x gene product in the regulation of cardiac mitochondrial function.

2211-Pos Board B348

Human Erythrocyte Mechano-Activated K^+ Channel A, a Kinetic Study of Intraburst Activity: Effect of Chlorpromazine

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Human red blood cells (hRBCs) have a mean life span of 120 days. However, little is known about this biological clock. We have presented evidence of the existence of the Human Erythrocyte Mechano-activated K^+ Channel A (HEMKCA), whose open probability (Po) depends on the pressure applied on the membrane. This channel shows a $PK^+/PNa^+ \approx 100$ with a mean conductance of 21.8 pS and it is modulated by Ca^{2+}(1)(2). We propose HEMKCA as the pressure sensor involved in the aging process of hRBCs at microcirculation level. Here we present a kinetic analysis for the HEMKCA burst activity in isolated membrane patches with 10 uM Ca^{2+}. In order to define the Tcrit we analyzed records with Po $\geq$ 0.8 (90s), partitioned into windows of 10s. These records were classified as "low" (Po$<$0.9) and "high" (Po$\geq$0.9) activity. To determine the rate constants, interval durations were fitted by their corresponding probability density functions, assuming a Markov scheme with dead time of 430 us. We found that "High" activity requires at least two closed states (tau1$=$0.26 $\pm$ 0.019; tau2$=$12.29 $\pm$ 1.43), whereas "low" activity requires at least three closed states (tau1$=$0.25 $\pm$ 0.017; tau2$=$12.35 $\pm$ 1.45; tau3$=$97.92 $\pm$ 25.92), with one state open in both cases